Coxsackie Viruses and Diabetes Mellitus

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Summary

Serum specimens from 162 patients with insulin-dependent diabetes of recent onset and 319 controls were tested for neutralizing antibodies to Coxsackie viruses types B1 to B5. Antibody to type B4 virus was more often found in diabetics than in controls, particularly in the 10-19 year age group. Though controls were not matched for geographical area it was thought that this was unlikely to explain the difference found. The month of onset of diabetes in the patients studied showed a pronounced seasonal incidence, which resembled that found in earlier studies.

Introduction

Reports of virus-induced diabetes in animals (Barboni and Manoccio, 1962; Craighead and McLane, 1968) have stimulated interest in the possibility that viral infection may be associated with the onset of diabetes in man, and the knowledge gained from animal models has focused attention on the picorna group of viruses. Evidence has been reported of higher titres to Coxsackie B4 virus in insulin-dependent diabetics seen within three months of onset (Gamble *et al.*, 1969), and changes have been observed in the pancreatic islets of an infant dying with Coxsackie B4 virus infection resembling those seen in children dying after the onset of acute diabetes (Newton, cited by Craighead, 1972). Morphological changes in the islets of mice after Coxsackie B virus infection have also been reported (Burch *et al.*, 1971; Harrison *et al.*, 1972; Coleman *et al.*, 1973) and Coxsackie B4 virus infection in mice may be followed by diabetes (Coleman *et al.*, 1973). This report describes further evidence of an association between the onset of insulin-dependent diabetes and infection with Coxsackie B4 virus in man, and observations on the seasonal variations in the onset of insulin-dependent diabetes.

Materials and Methods

Serum specimens were collected in nine centres in England and Wales from 162 patients with insulin-dependent diabetes of recent onset, between May 1970 and April 1973. A total of 319 control specimens were assembled from surplus sera submitted to the Epsom Public Health Laboratory for other purposes; some of these patients were ill, but those with syndromes resembling the common manifestations of Coxsackie virus infection were excluded. Two control specimens were collected for each patient, matched for age within five years and for date of collection within 28 days.

All specimens were tested for the presence of neutralizing antibody to Coxsackie virus types B1 to B5 at a titre of 1/4 by conventional techniques. Mixtures of serum diluted 1/4 and about 100 tissues culture infective doses of virus were incubated for 60 minutes at room temperature, and 0·1 ml aliquots were inoculated into Vero cell tissue culture tubes. The presence of neutralizing antibody was determined when virus control titrations showed appropriate cytopathic effects, and for maximum sensitivity minimal cytopathic effects affecting less than 5% of the cell sheet were ignored.

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Results

The average ages of the diabetic and control groups were 18·8 and 19·0 years respectively; controls were individually matched with cases for age within five years (mean ± 1·4 years) and time of collection within 28 days (mean ± 13·1 days). Controls were therefore closely matched for age and in time but as matching for geographical area had proved impossible they were people domiciled in south-east England. Analysis of the distribution of Coxsackie B virus neutralizing antibodies by area (table I) showed that in every area studied a higher proportion of diabetics had antibody to B4 virus than the controls. The possibility that some areas had experienced a higher incidence of Coxsackie B4 virus infection than the controls could not be excluded, but it seemed unlikely that this would have been confined to only one of the five virus types over a period of three years.

TABLE I—*Coxsackie B Virus Neutralizing Antibody by Geographical Area*

Source of Sera	No. Tested	Sera with Titre ⩾ 1/4 by Virus Type (%)				
		B1	B2	B3	B4	B5
Diabetics:						
S.E. London*	31	8 (26)	17 (55)	13 (42)	23 (74)	10 (32)
Epsom	22	7 (32)	7 (32)	10 (45)	14 (64)	8 (36)
Brighton	20	4 (20)	7 (35)	8 (40)	14 (70)	8 (40)
Chatham	10	2 (20)	4 (40)	5 (50)	6 (60)	3 (30)
Leicester	45	12 (27)	22 (49)	22 (49)	32 (71)	19 (42)
Cardiff	17	4 (24)	8 (47)	8 (47)	15 (88)	6 (35)
Manchester	14	3 (21)	8 (57)	8 (57)	10 (71)	7 (50)
Other	3	0	2	1	0	0
All areas	162	40 (25)	76 (47)	75 (46)	114 (70)†	61 (38)
Controls:						
Epsom	319	76 (24)	132 (41)	159 (50)	186 (58)	127 (40)

*Sera collected from cases seen at King's College Hospital, London.
†P <0·01.

The distribution of antibodies by age group (table II) showed that the excess of diabetics with antibody to type B4 virus was confined to patients over the age of 10 years. In the group aged 10-19 years 87% of diabetics were positive compared with 65% of controls (P <0·001) and 63% of patients over the age of 20 years were positive compared with 55% of controls (not significant). Of all the patients studied 70% had antibody to Coxsackie B4 virus against only 58% of controls (P <0·01). The occurrence of antibodies to other Coxsackie B virus types did not differ significantly between patients and controls but diabetics of 20 or more years of age had antibody to type B2 virus more often than their matched controls.

The duration of diabetic symptoms at the time of collection of serum specimens had little effect on the proportion of diabetic patients with antibodies to any of the five types of Coxsackie B virus (table III).

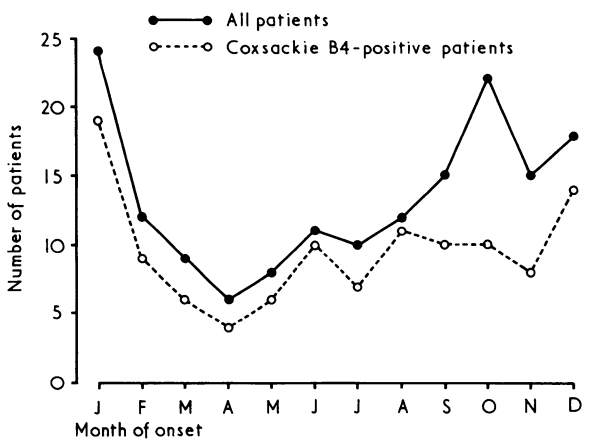
TABLE II—*Coxsackie B Virus Neutralizing Antibody by Age Group*

Age Group (Years)	No. Tested	Sera with Titre ≥ 1/4 by Virus Type (%)				
		B1	B2	B3	B4	B5
0-9	31	5 (16)	8 (26)	11 (35)	14 (45)	6 (19)
10-19	75	17 (23)	37(49)	36 (48)	65 (87)*	33 (44)
≥ 20	56	18 (32)	31 (55)	28 (50)	35 (63)	22 (39)
All ages	162	40 (25)	76 (47)	75 (46)	114 (70†)	61 (38)
Controls:						
0-9	62	12 (19)	20 (32)	19 (31)	30 (48)	19 (31)
10-19	149	31 (21)	67 (45)	86 (58)	97 (65)	63 (42)
≥ 20	108	33 (30)	45 (42)	54 (50)	59 (55)	45 (42)
All ages	319	76 (24)	132 (41)	159 (50)	186 (58)	127 (40)

*P <0·001.
†P <0·01.

TABLE III—*Coxsackie B Virus Neutralizing Antibody by Duration of Diabetes*

Duration of Diabetic Symptoms (Weeks)	No. Tested	Sera with Titre ≥ 1/4 by Virus Type (%)				
		B1	B2	B3	B4	B5
0-3	52	13 (25)	22 (42)	23 (44)	35 (67)	22 (42)
4-7	60	10 (17)	28 (47)	23 (38)	42 (70)	17 (28)
8-11	23	7 (30)	11 (48)	13 (57)	17 (74)	12 (52)
12-15	19	6 (32)	12 (63)	10 (53)	13 (68)	6 (32)
≥ 16	8	4 (50)	3 (38)	6 (75)	7 (88)	4 (50)
Total	162	40 (25)	76 (47)	75 (46)	114 (70)	61 (38)



Month of onset of diabetes in patients studied.

The month of onset of diabetic symptoms in the patients studied (see graph) showed a noticeable seasonal pattern, with a low incidence in April and peaks in October and January. Surprisingly, the October peak was not shown by those patients who had antibody to Coxsackie B4 virus and it was most pronounced in the patients who did not have antibody to this type. A larger sample would, however, be needed to say whether this was a constant phenomenon.

Discussion

Neutralizing antibody titres after viral infection generally reach a peak within a few weeks and then slowly decline, but they remain positive at low titres for many years. In a previous study (Gamble *et al.*, 1969) we found higher titres of Coxsackie B4 antibody in diabetics seen within three months of onset than in those with symptoms of longer duration. Since some juvenile diabetics have had symptoms for several months at the time of diagnosis, in the present investigation evidence of more remote infection was sought by testing for the presence of residual antibody at a low titre. With this procedure the duration of symptoms made little difference to the proportions of positive sera (table III). Since antibodies would persist for many years at this low level, one would expect that seasonal and geographical variations would have less influence on the data.

Patients and controls were well matched by age and date of serum collection, but matching for geographical area and social class was not possible and this must be borne in mind when assessing the findings. Nevertheless, the higher proportion of diabetics with Coxsackie B4 virus antibodies than that found in controls supports our earlier finding of an association between this virus and the onset of juvenile diabetes (Gamble *et al.*, 1969). The fact that the difference found was confined to type B4 virus antibodies and that it was shown for each area studied over a three-year period suggests that it was not due to localized outbreaks in particular areas. Moreover, the proportion of patients with Coxsackie B4 antibody in the south-eastern counties of Surrey, Kent, and Sussex was 57 out of 88 (65%), which did not differ significantly from that in the remaining areas—57 out of 79 (72%); in both cases the proportion was substantially greater than that found in controls (58%).

The absence of evidence of an excess of infection with Coxsackie B virus of any type in the diabetics under 10 years of age may seem surprising, and it is difficult to reconcile this finding with the known frequency of Coxsackie virus infection in children of this age. If diabetes were a direct consequence of Coxsackie B virus infection one would expect the incidence of juvenile diabetes to resemble that of other manifestations of Coxsackie virus infections with a high incidence of infection in the first few years of life, declining with increasing age. This suggests that susceptibility to virus-induced diabetes may be low at birth and increases with age—and there is some support for this from animal models—or that diabetes may result from cumulative damage to the pancreas from sequential viral infection. In very young children diabetes may be induced by infection with viruses other than those of the Coxsackie group; mumps virus is a possible candidate and further investigation of this is in progress.

The patients in this survey were clearly a small and selected sample and the seasonal pattern in the onset of their diabetes may not conform to the general pattern. It does, however, bear a remarkable resemblance to the seasonal incidence reported by Adams (1926) and by Gamble and Taylor (1969). The absence of an October peak in the patients with Coxsackie B4 virus antibody is surprising in view of the seasonal incidence of Coxsackie virus infection, which regularly has its peak in the autumn. The excess of diabetics with Coxsackie B4 antibody was present in every month except October and November, though numbers were too small to show any clear distribution pattern among the remaining months. If this finding is true for diabetes in general, it is incompatible with the idea that the disease is an immediate sequel to Coxsackie virus infection. If diabetes were the direct result of viral destruction of beta-cells, or of a process associated with the appearance of primary viral antibody, its peak incidence would occur in September or October, as happens with poliomyelitis, and the excess of diabetics with Coxsackie B4 antibody would be confined to those months. A process associated with the appearance of secondary or anamnestic antibody would be provoked by infection with another Coxsackie B or related virus and would have a similar seasonal incidence. Little is known about cell-mediated immunity in enterovirus infection but if diabetes were related to the development of delayed hypersensitivity after Coxsackie virus infection one might expect the seasonal peak of infection to be reflected in the incidence of diabetes, delayed perhaps by a short interval for the development of hypersensitivity. Though the incidence of diabetes does not accord entirely with this prediction, delayed hypersensitivity may yet prove to be concerned in some way. The lymphocytic infiltration seen in the islets of children dying with acute diabetes could be associated with cell-mediated immunity and Nerup *et al.* (1971) reported cellular hypersensitivity against extracts of porcine pancreas in diabetic patients by the leucocyte migration inhibition test.

If the seasonal pattern of diabetes is not attributable to Cox-

sackie or related virus infections other seasonal factors or combinations of factors must be responsible, and these may be viral, non-viral, or both. In this case the role of Coxsackie virus would appear to be that of an initiating factor, producing subclinical islet cell damage in patients in whom diabetes is subsequently precipitated by other seasonal factors. The results of our antibody studies throw little light on this problem as neither the present study nor our previous results indicate when Coxsackie virus infection occurred in relation to the onset of diabetes.

If viruses do induce diabetes in man, the question arises as to what proportion of the total incidence of diabetes is associated with Coxsackie virus infection. There are at present few indications that maturity-onset diabetes is associated with viral infection, but in our previous investigations (Gamble *et al.*, 1969) we found higher Coxsackie B4 virus antibody titres in non-insulin-dependent diabetics of acute onset than in controls. The numbers were too small for a significant difference to be found but the difference was of the same order as that found in the insulin-dependent diabetics and this type of patient merits further study. Of insulin-dependent diabetes, our results suggest that a substantial proportion may be associated with Coxsackie B virus infection and, if this is so, a search for a viral aetiology in the remainder would clearly be indicated. The numbers studied to date have been too small to show whether other members of the Coxsackie B groups are implicated but it is unlikely that they could account for many cases without it being apparent from our results. Other picornaviruses are obvious candidates, particularly in cases occurring in autumn, in whom we found no excess of Coxsackie virus antibodies. Mumps virus is not, however, a member of the picorna group and the possibility that other groups of virus may be involved should not be overlooked, particularly in younger children.

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Alteration of Bile Salt Metabolism by Dietary Fibre (Bran)

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Summary

Feeding dietary fibre in the form of bran induced changes in bile salt metabolism in five people with intact gall bladders. There was evidence of reduced dehydroxylation of bile salts; the proportion of deoxycholate conjugates in bile was reduced and the transfer of radioactivity from labelled taurocholate to deoxycholate was decreased. These findings, which were independent of changes in intestinal transit rate, imply that bran reduced the degradation of bile salts by colonic bacteria. This property of bran accords with recent theories that fibre-depleted diets favour the degradation of bile salts in the colon. These findings may be relevant to the aetiology of large bowel cancer.

Introduction

There is increasing interest in the possibility that fibre-depleted foods (especially white flour and sugar) are concerned in the causation of various diseases of modern civilization (Cleave *et al.*, 1969; Burkitt, 1971, 1973; Heaton, 1973 a). With two of these diseases, carcinoma of the colon and gall stones, it has been suggested that degradation of bile salts by colonic bacteria may play a pathogenetic part (Burkitt, 1971; Hill *et al.*, 1971; Heaton, 1972). Burkitt (1971) claimed that degradation of bile salts is favoured by a low-residue, fibre-depleted diet. Evidence is, however, lacking for this speculation.

Bran is the fibre-rich fraction of wheat which is discarded in the milling of white flour. It is known to influence the motility of the intestine (Cummings, 1973; Harvey *et al.*, 1973), while diets rich in mixed cereal and vegetable fibre cause increased faecal excretion of calories, fat, and nitrogen (Southgate and Durnin, 1970). In vitro, fibre has bile salt binding properties (Eastwood and Hamilton, 1968). Nevertheless, the effects of fibre on bile salt metabolism have not been reported except for conflicting statements that faecal bile acid excretion is or is not increased by a raised intake of cellulose (Shurpalekar *et al.*, 1971; Stanley *et al.*, 1972; Eastwood *et al.*, 1973), bran (Eastwood *et al.*, 1973), or mixed fibre (Antonis and Bersohn, 1962). We have studied the effect of bran on bile salt metabolism using radioactively labelled bile salts and analysing duodenal bile.

Subjects and Methods

The subjects studied were three healthy women with normal cholecystograms, two women with symptomless gall stones in functioning gall bladders, and six women who had undergone cholecystectomy but were otherwise in good health. All were volunteers who had given informed consent. All 11 subjects were given a tracer dose of 3 to 5 μCi radioactive bile salt intravenously, and on each of the next four mornings bile was aspirated from the duodenum. Subjects with intact gall bladders were given cholecystokinin to stimulate

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